

## CHANGES IN ANTENNAL SEGMENTATION DURING THE NYMPHAL DEVELOPMENT OF BRITISH GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE)

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### ABSTRACT

The mode of increase in the number of antennal segments during nymphal development common to ten species of British grasshoppers is described and shown to correspond with that recorded by others for a range of non-British examples. This provides a theoretical background to the system used previously for deriving numerical nymphal stages from antennal segmentation in two species, and forms the basis of a simpler procedure now proposed for this purpose. Enumeration of a stage by the means described reveals, for an adult, the number of stages through which it has passed and, for nymphs of the last two instars, the total number required to complete development; in combination with other features it may provide a useful indication of this for certain nymphs at one stage earlier.

### INTRODUCTION

A typical feature of the nymphal development of grasshoppers is the increase in the number of antennal segments that accompanies each moult. Should these changes follow a predictable pattern a count of the segments would afford a useful addition to the other characters normally enlisted to indicate the serial number of the stage attained. The upsurge of attention to the locust problem in the early years of the last century gave impetus to the recording of such counts, so that Uvarov (1928) was able to produce a table, compiled from various sources, of the antennal segmentation of successive stages of eight named locust species. Two aspects of these counts stand out: their close similarity, stage by stage, for most of the species included; and their variability which, although small, often involved listing as ranges. Uvarov also commented that in numerous counts for *Locusta migratoria* (L.) Lebedeva (1925) had found some deviations from the averages shown in the table, so that identification of a stage by this means alone could be liable to error.

Although counts of antennal segments continued to be recorded, this variability caused their potential value for this purpose to be rather under-explored. Uvarov (1928) had also warned that such counts could be difficult to determine as some segments were often very imperfectly separated. A few counts introduced in studies of modified developmental histories of some locust species (e.g. Burnett, 1951; Remaudière, 1954) lent general support to conclusions based largely on other characters, but views differed on whether, for inclusion in the totals, these poorly-separated segments should be regarded as single or double. A notable contribution had already been made by Michelmores and Allan (1934) who showed that, for the Red-Winged Locust *Nomadacris septemfasciata* (Serv.), appending to the total count a note of the position of these rudimentary sutures permitted the recognition of the stage of any hopper with certainty. The possible significance of the linking of these two variable aspects of segmentation appears to have received no further attention at the time.

While these counts were being recorded some efforts were made to determine the manner in which the "new" segments originated. Uvarov (1928) reproduced

a diagram of Takahashi (1925) showing for *L. migratoria* how the segments of each stage became divided to give the increased number of the next. Paoli (1937) gave a similar scheme in tabular form for the Moroccan Locust, *Dociopterus maroccanus* Thunb. Both schemes show that, for these species, the two basal segments (scape and pedicel) and the five apical segments of the first nymphal stage are not involved in the subsequent segmental divisions. The agreement between these was noted by Santoro and Carames (1973) who in turn gave a detailed and clearly presented account of a similar process for twelve South American species, differing only in that the number of unaffected apical segments was less than five in some of these.

As regards British grasshoppers, information of this nature has been slow to emerge. The first list of segmental counts for almost all nymphal stages of both sexes of ten species was produced by Richards and Waloff (1954). Here again the broad equivalence between the figures for a particular stage of the different species is apparent, as also is the need for the counts to be expressed as ranges, sometimes of as many as four or five consecutive figures. There must be at least a suspicion that the spread of seven figures for "Instar II" of female *Chorthippus brunneus* (Thunb.), showing a hint of two maxima, could have resulted from confusion between stages two and three.

A range of counts for 30 individuals of each sex for the first three or four stages of *C. brunneus* was given by Hassall and Grayson (1987). Counts for the later nymphal stages and for adults of *C. brunneus* (Collins, 2001), and a few for the full development of *Stethophyma grossum* (L.) (Collins, 2003) were used to ascribe serial numbers to the stages of these species. This was based on a system recognising that the variation resulted from the position as well as the presence of incompletely separated segments, but presented without explanation. The theoretical background to this is shown here to be provided by the manner in which new segments originate at each moult by subdivision of those of the preceding instar.

## EXPERIMENTAL

All ten species of Acrididae currently accepted as resident in mainland Britain (see Marshall & Haes, 1988) have been reared from eggs laid in captivity and, except for *Omocestus rufipes* (Zett.), from first-stage nymphs taken in the field. These were reared individually under conditions similar to those described previously for *C. brunneus* (Collins, 2001). Details of the antennal segmentation were recorded for all instars and for the resulting adults, and supplemented by similar data for a full range of wild-caught examples. Additionally, complete sequences of the antennal sheaths detached from nymphal casts of both sexes of reared individuals were prepared for microscopic examination and the changes in segmentation for all species found to reproduce those seen in the living insects. While numerous examples of the more abundant species have been examined in this way, the numbers available have of necessity been reduced for those less widely distributed. For *Chorthippus vagans* (Eversmann) in particular, only about 20 individuals, including those bred in captivity, were reared. Most samples were taken in south-east England, and all south of the 300km National Grid line. For all specimens available the growth processes described below were found consistently in both sexes of all ten species.

## RESULTS

The antennae of first-stage nymphs consist of a scape, a pedicel, and a flagellum of 11 segments, of which the first and sixth are the longest while the second to the

sixth show an overall, although often not quite regular, increase in length (Fig. 1). At the inner apical corner of the seventh is a specialised area, usually recognisable at low magnification in the living insect as a small blister-like protuberance, somewhat smoother than the background and often differing slightly in colour (Collins, 1992). This retains its position relative to the apex throughout development, and may consequently provide a useful marker for the five apical segments which, with the scape and pedicel, are not involved in the production of new segments as growth proceeds (Fig. 2). For the subsequent stages therefore the changes that occur may be related to flagellar segments 1 to 6 of the first stage. In accordance with the nomenclature of Santoro and Carames (1973) the terms "basal segment" will be applied to the first of these, "basal group" to all segments subsequently derived from this, and "medial" to segments 2 to 6 and those derived from them.

The somewhat complex appearance of the schemes shown by Takahashi (1925) and Paoli (1937) is considerably simplified when recognised as presenting two distinct processes occurring simultaneously. At each moult one new segment is split off from the apex of the basal segment while, at the same time, a sequence of subdivision of each segment into two, and no more, beginning at segment 6 of the first instar moves progressively down the flagellum towards the base. At the first moult, segments 1 and 6 both become divided into two, while new divisions appear on segments 5, 4 and sometimes 3, becoming progressively weaker as the base is approached. Even on segments 1 and 6 the new divisions are recognisable as being slightly weaker than those of the first stage, and by causing almost no indentation of the side margins, while all the new divisions leave the relative lengths of the pre-existing segments virtually unchanged (Fig. 1).

Examination of the cast skins provides confirmatory evidence for the sites of the new segments from the distribution of the coeloconic sensillae. In the first instar two of these are normally present on the underside of segments 2 and 4 (although one or both may be missing from either segment or, very occasionally, an additional one may be present), while segments 1, 3 and 5 have none. Segment 6 bears a larger and rather variable number. In the second stage the subdivision of segment 1 causes segment 2 to move up into third place, accompanied by its coeloconic sensillae, while that of segment 6 carries the coeloconic sensillae into the new apical portion, leaving the basal portion with none. Moreover, at the first appearance of subdivision of each segment downwards from 6 in this and the later instars, any coeloconic sensillae present pass without change into the new apical portion. The change for segment 4 at the first moult may therefore reflect both these effects, the same movement up the antenna shown by segment 2, usually accompanied by the subdivision passing down (Fig. 1).

From the third instar onwards the divisions resulting from the doubling process passing down the antenna no longer show the gradual decrease in intensity noted for the second stage. Nonetheless, the advance often results in only a partial subdivision of the segment at the leading edge; at the following moult this division becomes complete while the next segment down shows the partial subdivision. Because this subdivision can show all gradations between being scarcely visible and being almost as definite as the others the segment involved may be referred to as the "doubtful" segment. Occasionally two adjacent segments show this effect, one having a fairly marked subdivision and the other only a faint indication of this. Eventually, and usually in the third or fourth instar, the foremost segment of the basal group moving up the antenna meets the front of the doubling process passing down and becomes the doubtful segment. While the progress of the former is regular and predictable the

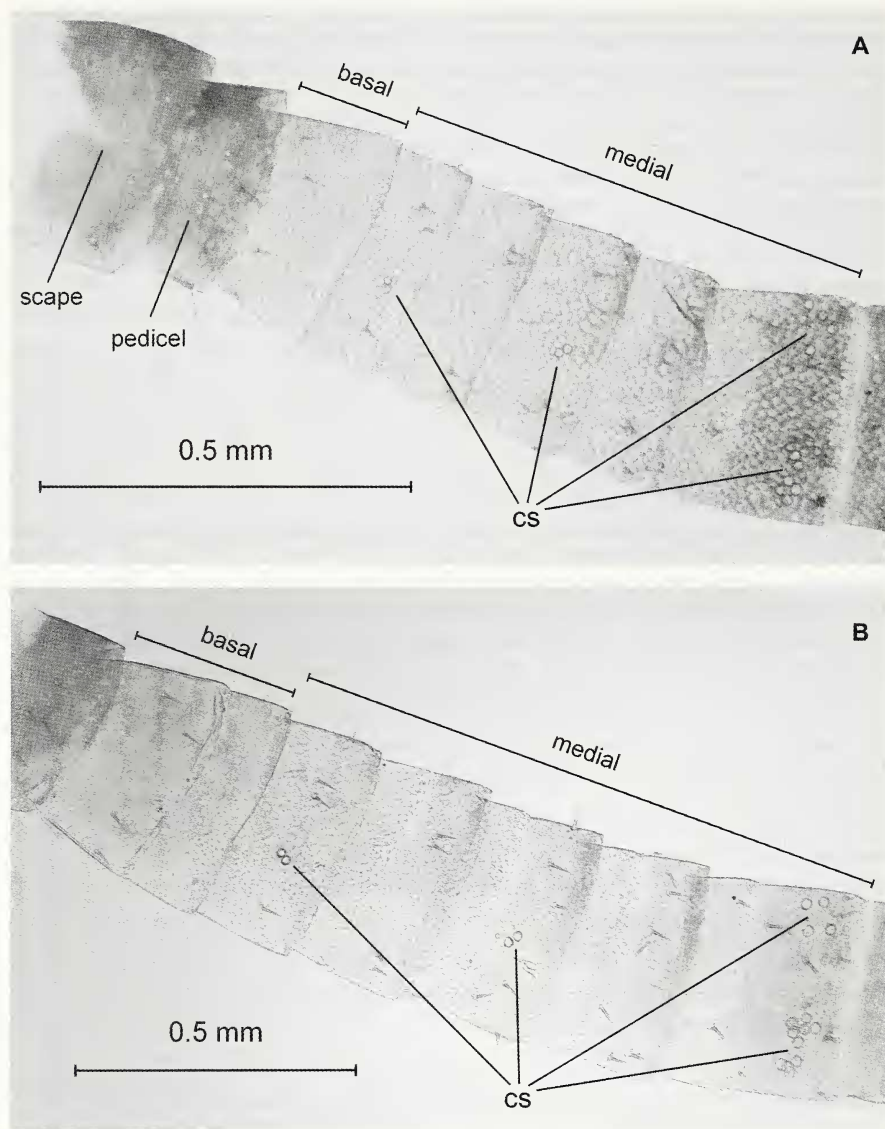


Fig. 1A & B. Basal and medial portions of the first two antennal casts from a nymphal sequence of female *Stethophyma grossum*. The sites of the new segments in the second (B) are shown by the resulting displacement of those bearing the coeloconic sensillae (cs).

rate of advance of the latter can vary to some extent from moult to moult and between individuals. When, as often happens, the advance of the doubling front proceeds at one segment per moult the site of the doubtful segment remains constant relative to the flagellar base, and for many individuals tends to be in the third or fourth position.



As the stages progress, each successive new segment of the basal group gradually lengthens as it passes up the antenna so that, in the absence of further subdivision, these segments would form a sequence, extending to at least the first of the medial series, showing a regular increase in length. The effect of the downward subdivision however is the production of two new segments, each only slightly more than half as long as that from which it was derived. While the doubtful segment, if regarded as single, conforms to the regular increase in length, and is longer than the adjacent component of the fully-divided segment beyond, if regarded as divided into two, each new portion is shorter than the undivided segment immediately nearer the base. This sudden change in regularity is usually very noticeable in nymphs (Fig. 2). In adults,

Total count, based on Mason's notation.	A 19 (3+4)	Reduced count, equivalent to the original 5 medial segments, plus 3 from the basal group.	A } 8
Sum, as defined in text, 22 for each.	B 18 (4+5)		B } 8
	C 17 (5+6)		C } 8

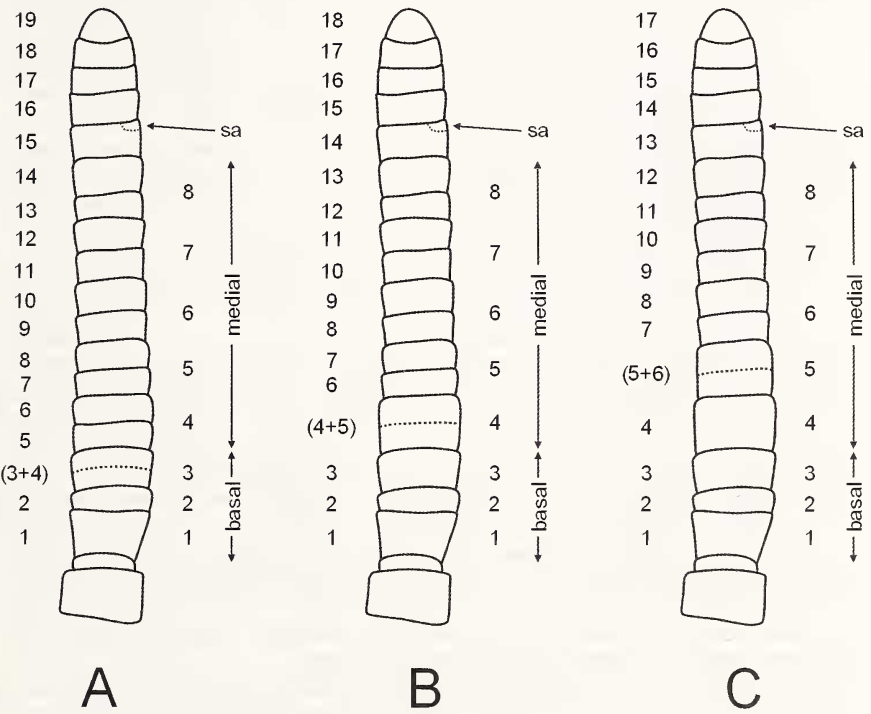


Fig. 2. Segmentation (diagrammatic) of the left antenna of a third instar acridid. (A) showing partial subdivision of the flagellar segment in the third position, (B) in the fourth and (C) in the fifth. All three show the site of the specialised area (sa), and have the total flagellar count on the left. The reduced count, shown on the right, is the same for A, B or C, whichever segment is regarded as the doubtful.

particularly in males, the marked increase in length of the antennae at the final moult may lead to a succession of mid-antennal segments having much the same length. This does not usually affect the relative lengths in the region where the doubtful segment is likely to occur, although it may reduce the contrast between them.

## DISCUSSION

For all ten British species the manner of increase in the number of antennal segments follows the same basic pattern as that already described by others for several non-British species. This permits the assessment of the order of any nymph in the developmental sequence and, for adults, the number of stages through which they have passed. The regular production from the basal segment of one new segment per moult would provide an immediate indication of the stage attained were it not for the complication introduced by the advance of the doubling front. Recognition of the doubtful segment serves to show how far this has reached, and thus provides a means of allowing for it. This was the background to the empirical procedure used earlier for this purpose (Collins, 2001, 2003). In a modification of the notation introduced by Mason (1954), the sequential numbers of the two partially fused segments were included in brackets after the total flagellar count. Addition of the first of these numbers to the total count yielded a figure that was constant for, and typical of, any particular instar. This addition of a positional number to a total that already includes it is closely equivalent to regarding all the basal and medial segments as having been doubled, thus eliminating the variable aspect. For the first instar, with as yet no doubtful segment, this system is not applicable; for the second the sum is found to be 20 and then to increase by 2 at each subsequent moult up to a total of 28 for an adult that has passed through five nymphal stages. Disadvantages of this procedure are that it involves counting all the flagellar segments when, for at least the later instars, it is easy to "lose count", and that it leads to a figure with no immediate physical significance.

A simpler alternative procedure involves counting the flagellar segments from the base until the doubtful segment is reached, counting this, and continuing to count each pair beyond, as one, and ceasing as soon as the apical five are reached. This effectively reduces the count to a total that would have been obtained for the medial and basal group segments had no doubling occurred. As the former is constant at five, subtraction of this from the reduced count yields a figure equal to the number of the stage. With a little practice this method of counting becomes much easier than the description would suggest, the eye automatically adjusting to the change from singles to pairs as soon as the doubtful segment is reached. If more than one segment shows incipient subdivision some uncertainty may remain as to which should be regarded as the doubtful. In this method of counting, as indeed in the other, so long as the gradual increase in length shown by the first few single segments and then by pairs is recognised it does not matter unduly which segment is so accepted. This can be appreciated by reference to Fig. 2, where the same reduced count results whichever segment is shown as incompletely subdivided.

Occasionally no obvious doubtful segment as defined by a weaker subdivision is observed, all intersegmental divisions appearing equally strong. Should this be accompanied by the lack of contrast in lengths mentioned earlier the result may be a short sequence of segments of almost equal length, which in turn makes it unclear as to when counting in pairs should commence. An error here will normally lead to the presence of a single segment "over" at the end of the count; counting downwards in pairs from the apical five will usually give a clue as to the correct interpretation.

For living or recently dead specimens, barring damage or deformity, there is normally little difficulty in recognising the segmentation and disregarding the five apical segments. Preserved material may present more of a problem. In spirit-stored nymphs the antennal contents often shrink away from the outer walls, thus confusing the position of the segmental divisions. This difficulty is compounded by the fact that the shrunken inner matter probably exhibits the segmentation of the next instar, so that the divisions no longer correspond either in position or number. In pinned dried adults shrivelling of the antennal tips and a tendency to acquire a surface bloom can obscure both the divisions and the specialised area, making identification of the apical five difficult. Repeated moistening of the tip may help to reveal the segmentation; otherwise some clue may be afforded by the relative lengths of the regions involved. Enumeration by the reduced method will normally lead for adults to a count of 10 or 11; if, when a count of 10 has been reached, the remaining apical portion is less than twice as long as the last pair counted, this count is probably correct. If the remaining apical portion is twice, or rather more, than as long as this the presence of a further doublet giving a count of 11 should be suspected. For both types of problem, removal of the antenna for microscopic examination after clearing with aqueous alkali may be the only solution.

The study of locusts referred to above had shown that variable instar numbers within certain species could be a regular feature of their development, and was associated in some with phase change. Early views on this aspect were well summarised by Albrecht (1955). It is now clear that such variability can also occur in some British species, where it is usually restricted to females. While the males of these, except *S. grossum*, seem normally to have four nymphal instars, females of *C. brunneus* may pass through four or five (Hassall & Grayson, 1987; Collins, 2001) and similar effects, leading to reduced flagellar counts for adult females of 10 or 11 respectively, have been observed in certain other species (Collins, unpublished). Further investigation into potential causes underlying this variability requires an unambiguous means of determining the numerical order of any nymph within a moulting sequence so as to recognise the possible developmental types as they may be encountered.

Serial numbers are often assigned to nymphal stages on the evidence of qualitative characters such as the degree of development of wing-rudiments and external genitalia, or of dimensions, such as hind-femora lengths, that are subject to a range of individual variation. These can only be assessed by comparison with corresponding features in nymphs of known or assumed history, and may show differences too subtle for distinguishing between instars that are members of development sequences differing in the total number of stages. The interpretation of flagellar segmentation described here provides a quantitative approach that requires no previous knowledge of the stages involved, and offers the only external feature so far observed for distinguishing between adults having different nymphal histories.

On the assumption that the last two instars may be recognised by the possession of reversed wing-rudiments (Uvarov, 1966) and distinguished by the degree of development of these, assessment by antennal segmentation of their numerical stage provides an indication of the number of nymphal stages required for their full development. Even before wing-reversal some nymphs that have hind-femora dimensions that fall within or close to quoted ranges for Instar II may show antennal segmentation typical of the third instar. These may be regarded as requiring a total of five nymphal stages for completion of development. In studies designed to monitor nymphal progress within a population, or to examine such variability in development as may be influenced by differences in site characteristics, this approach should offer a simple, rapid and probably more reliable means for judging these aspects.

## ACKNOWLEDGEMENTS

The author is indebted to the late Dr. N. Jago for calling his attention to the publication of Santoro and Carames, whose valuable contribution he would not otherwise have encountered. Particular thanks are also due to my son, Graham A. Collins, for his painstaking preparation of the figures in a form suitable for reproduction.

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